Review article

Immunotoxicology: suppressive and stimulatory effects of drugs and environmental chemicals on the immune system

A discussion*

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Abstract. The fundamental characteristic of the adaptive immune system which has evolved in the vertebrates is the ability to recognise, and subsequently destroy, "foreign", and potentially harmful, antigens. The selective advantage which the immune system confers is the capacity to resist infectious, and possibly malignant, disease. It has been apparent for many years that individuals in whom immune function is impaired, due either to a congenital defect or to other factors such as treatment with certain immunosuppressive drugs, exhibit an increased susceptibility to infection and, in some cases, an elevated risk of developing at least some forms of malignancy. There is an increasing awareness from rodent studies that a variety of drugs and environmental chemicals have the potential to unintentionally impair components of the immune system. Risk assessment, based upon data from chemically induced changes in one or more parameters of immune function, is, however, dependent upon a knowledge of the functional reserve of the immune system. One of the objectives of the meeting from which this report derives was to examine what sources of information are available, and what experimental protocols can be employed, to permit accurate evaluation of immunological reserve. Although, under normal circumstances, the immune system selectively and specifically recognises foreign antigen, it is clear that the potential to recognise "self" is present and that in certain circumstances this potential is realised. Antibodies directed against normal tissue antigens have been shown to be associated with, and in some instances the presumptive cause of, "autoimmune" disease. There is a growing list of drugs and chemicals which are capable of eliciting autoantibodies and pathological autoimmune reactions. A second purpose of this meeting and of this report was to review the current state of knowledge regarding drug- and chemical-induced autoimmunity.

Key words: Autoimmunity – Environmental chemicals – Drugs – Immunosuppression – Immunostimulation

Introduction

There is a growing awareness that a variety of chemicals have the potential to influence the functional activity of

the immune system. Induced changes in immunological status can be broadly divided into those in which immune function is impaired and those in which tissue-damaging allergic or autoimmune responses are initiated. The purpose of the meeting from which this report is derived was two-fold, firstly to examine what sources of information are available for determining the relationship between impairment of immune function and altered host resistance to infectious and/or malignant disease, and secondly to explore the current state of knowledge regarding the potential of drugs and chemicals to induce autoimmunity.

Chemically-induced impairment of immune function

During the last decade the field of immunotoxicology has attracted considerable attention and there now exists substantial literature, a review of which reveals that a variety of chemicals are able to impair the functional integrity of the immune system of rodents. Evaluation of the toxicological significance and human health implications of data obtained from experimental studies in rodents requires, however, a careful consideration of a number of factors. From a purely practical point of view it is relevant to consider whether there exist chemicals which, under certain conditions of exposure, are selectively immunotoxic, and would therefore escape detection in routine toxicological analyses. Although it is not unreasonable to suppose that selective immunotoxins exist, there are few examples in the literature. It is also pertinent to address the question of whether perturbations in immune function are reversible, and here again little information is available. Perhaps the major problem posed by experimental immunotoxicity studies is the relevance of quantitative or qualitative changes in one or more components of the immune system to the functional activity of the integrated mechanisms of host defence and the ability to resist infection and malignant disease. Of direct relevance to the relationship between empirical observations of dysfunction following chemical exposure and the integrity of host defence mechanisms is the extent to which the immune system possesses a functional reserve. There are two avenues of investigation which might be expected to yield information of value in assessing the functional reserve of the immune system. Firstly, in experimental systems, it is possible to examine directly the relationship between chemically induced perturbations of immune function and the ability to resist challenge with transplantable tumours and/or pathogenic micro-organisms. A second, and in our opinion, currently under-valued, source of information is available from the clinical and laboratory examination of congenital and acquired immune deficiency disorders in man.

The immune system as a target for toxicity

The ability of an organism to respond to foreign material is phylogenetically ancient and can be traced back to the protozoa and coelenterates. Such mechanisms of host resistance clearly offer important evolutionary advantages and in man there exist a number of "natural" (non-specific) defence mechanisms, such as the action of scavenger phagocytic cells, which play an important role in resistance to infectious disease. In addition to such natural protective mechanisms there has, in the vertebrates, evolved an exquisitely sensitive adaptive immune system in which there is a specific recognition of, and response to, foreign

antigens. The cardinal features of the mammalian adaptive immune system are memory, specificity and the capacity to distinguish between self and non-self. It is the lymphocyte which plays a pivotal role in adaptive immune responses and which exhibits the properties of antigenic specificity, immunological memory and the ability to distinguish foreign, and potentially harmful, antigens from those normally expressed in the organism. Lymphocytes are clonally distributed with respect to antigenic specificity and each clone of lymphocytes possesses a unique membrane receptor for antigen. On primary exposure to foreign material those clones of lymphocytes which express complementary membrane receptors recognise and respond to antigenic determinants. The response comprises both division and differentiation. The induced prolifera-
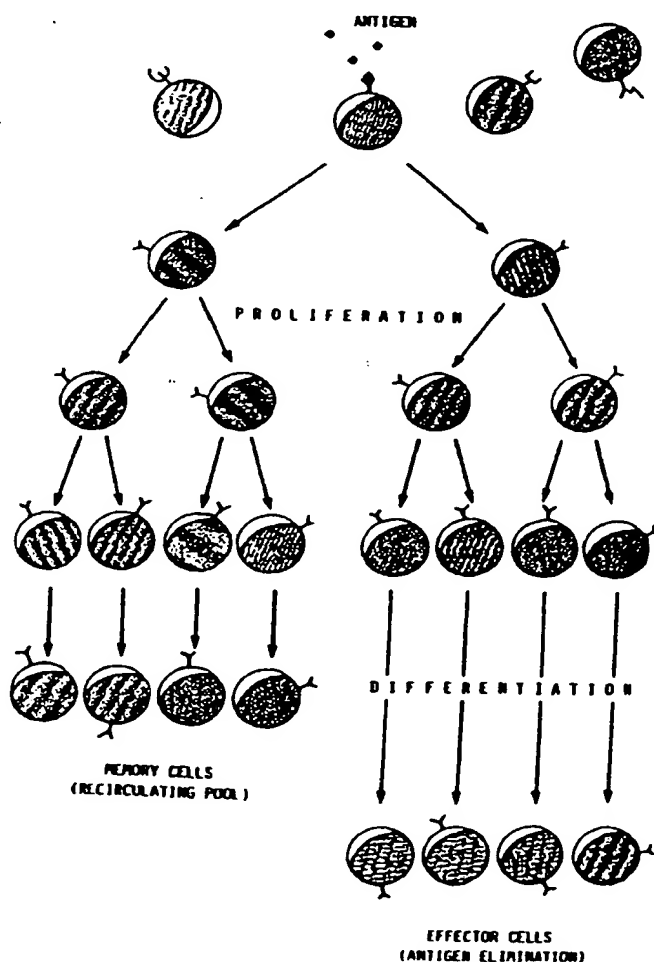
Fig. 1. Exposure to antigen results in the stimulation of those clones of lymphocytes (both T and B cells) which express complementary membrane receptors for antigen (clonal selection). Antigenic stimulation results in division (clonal expansion). A proportion of daughter lymphocytes differentiate into cells which either directly, or through the elaboration of effector molecules, eliminate antigen. The remainder of daughter lymphocytes fail to differentiate and constitute an expanded pool of cells (memory cells) which facilitate a more pronounced response following subsequent exposure to the same antigen

tion results in an expansion of those lymphocyte clones capable of recognising the stimulating antigen. Following division, a proportion of daughter lymphocytes undergo terminal differentiation into effector cells whose physiological role it is to eliminate antigen. The remaining daughter cells fail to differentiate and comprise the long-lived memory cells which provide an increased pool of antigen-sensitive lymphocytes that mount an accelerated and more aggressive immune response following secondary exposure to the same antigen (Fig. 1).

Superimposed upon the complexity of clonal distribution is the functional heterogeneity of lymphocyte populations. The two main classes of lymphocyte involved in adaptive immune responses are T and B lymphocytes. Following antigenic stimulation, B lymphocytes differentiate into plasma cells which possess the synthetic and secretory machinery to manufacture and export large amounts of antibody which has a specificity identical to that of the membrane antigen receptor expressed on the stimulated B lymphocyte. Antibodies are of particular importance in host resistance to extracellular bacterial infection. The effector cells into which antigen-stimulated T lymphocytes differentiate are those which are able to recognise and lyse infected host cells and those which elaborate a variety of soluble factors, known collectively as lymphokines. Lymphokines are responsible for a variety of important immune phenomena, including intercellular communication and the recruitment and activation of phagocytic cells. Effector T lymphocytes play a central role in resistance to infection by viruses and facultative intracellular bacteria where the effectiveness of antibody molecules is limited.

The last 20 years have witnessed significant and exciting advances in our understanding of the mechanism of action and control of immune responses, and it is now apparent that normal immunological function is dependent upon a series of sophisticated interactions at the molecular, cellular and tissue level. Thus, for instance, it became clear in the early 70s that functional sub-populations of T lymphocytes can be induced which promote (known as T helper cells) and regulate (T suppressor cells) immune responses. It is apparent that, in addition to providing mechanisms for the recognition and elimination of foreign antigens, the immune system incorporates a variety of elaborate internal checks and balances which together allow for efficient homeostasis.

A detailed account of the complex cellular and molecular events which together comprise normal immune function and control is clearly beyond the scope of this review. The important point is, however, that, in common with all finely-tuned biological systems, immune activity is potentially sensitive to disruption of normal function either through physiological or pathological change or following exposure to xenobiotics. One can envisage that relatively subtle changes in the delicate molecular and cellular fabric of immunity could create a functional imbalance causing a cascade of perturbations resulting in marked alterations in physiological function.

The importance of a functionally intact adaptive immune system is most clearly illustrated by the clinical consequences of severe congenital and acquired immune deficiency disorders in which one or more components of the immune system are affected. In the context of immunotoxicology it is relevant to consider whether the natural history of human immunodeficiency disorders provides in-

formation of value in assessing the functional reserve of the immune system.

Congenital and acquired immune deficiency disorders in man

The spectrum of immune deficiency disorders range from relatively minor changes such as the absence of an immunoglobulin class or sub-class to severe combined immunodeficiency which is characterised by the failure of T and B lymphocyte development and is immediately life-threatening (Table 1).

The primary manifestation of immunodeficiency is undue susceptibility to infections; infections that are more serious, more persistent, more unusual or recur more frequently than in the normal population. Infections in immunodeficiency states are characteristically related to the nature of the lesion. For instance, antibody deficiencies are typically associated with gram-positive bacterial infections, while patients with cellular immune deficiencies are

Table 1. Congenital and acquired immunodeficiency disorders[a]

A. Selected examples of congenital immunodeficiency disorders

Disorder	Immunological defect	Primary symptoms
Severe combined immunodeficiency disease (SCID) (Glanzmann and Rinicker 1950; Gitlin et al. 1964)	Lack of T and B lymphocytes	Multiple viral, bacterial, fungal and protozoal infections. Patients usually succumb within the first 12 months of life
Congenital thymic aplasia (DiGeorge syndrome) (DiGeorge 1968)	Thymus absent or small, T lymphocytes absent or reduced in number	Recurrent or chronic infection with viral, bacterial, fungal or protozoal pathogens
X-linked hypogammaglobulinaemia (Bruton's disease) (Bruton 1952)	Absence of B lymphocytes, Hypogammaglobulinaemia	Onset of recurrent bacterial infection following decay of transplacental maternal immunoglobulin
Chronic granulomatous disease	Defective intracellular killing of micro-organisms by phagocytes	Chronic and acute bacterial and fungal infection

B. Selected causes of acquired immunodeficiency

Protein loss	protein-losing enteropathy, nephrotic syndrome, protein hypercatabolism
Malignancy	especially Hodgkin's disease, leukaemias and myeloma
Drugs	steroids, alkylating agents (cyclophosphamide, chlorambucil), purine antagonists (6-mercaptopurine, azathioprine), cyclosporin A
Viral infection	measles, EBV. HIV
Surgery/trauma Malnutrition Ageing	

[a] Primary sources: Fudenberg et al. 1980; Webster 1983; Chapel and Haeney 1984

susceptible to mycobacterial, protozoan, fungal, viral and opportunistic bacterial infections. Defects of phagocyte function commonly present as infections caused by staphylococci, fungi and gram-negative bacteria, while some complement disorders predispose the individual to neisserial infections. Specific infections are sometimes associated with particular forms of immunodeficiency. Thus, for instance, ECHO virus encephalitis is common in hypogammaglobulinaemia and progressive Epstein-Barr virus infection in X-linked lymphoproliferative disease. Patients with immunodeficiency also exhibit an increased incidence of malignant disease. Malignancy, usually of lymphoreticular origin, has been reported in about 5-15% of patients with certain forms of immune defects (Penn 1977, 1985).

Correlation of clinical symptoms with plasma immunoglobulin concentrations or with the frequency and functional activity of T lymphocytes suggests that a relatively severe immune deficit (<60% of control values) is necessary for significant changes in the integrity of host resistance.

At present there is a lack of well-documented correlations in man between quantitative or qualitative changes in immune function and the appearance of clinical signs of immunodeficiency. It is therefore necessary to examine whether experimental animal studies provide more detailed information about immunological reserve and whether this information is of value in human risk assessment.

Experimental studies of immunotoxicity

The catalogue of chemicals which have been reported to influence the integrity of immune function is continually growing (Descotes 1988). Only a relatively small number of such chemicals have, however, been the subject of exhaustive investigation. To illustrate the issues raised in this review it is necessary only to consider examples from each of three chemical classes which have received more attention than most: the polyhalogenated hydrocarbons, the organotins and heavy metals.

Polyhalogenated hydrocarbons. A number of polyhalogenated hydrocarbons (PHH) have inadvertently become widespread environmental pollutants and their inherent lipophilicity and low biodegradability result in their concentration within the food chain, including human milk. There are numerous reports that chemicals within this group, notably the polychlorinated biphenyls (PCB) (Vos and Van Driel-Grootenhuis 1972; Loose et al. 1978; Thomas and Hindsill 1978) the polybrominated biphenyls (PBB) (Luster et al. 1978b, 1980; Fraker 1979) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Vos et al. 1973, 1978; Sharma et al. 1978; Hindsill et al. 1980) are, under the appropriate conditions of dose and exposure, associated with immunotoxicity in experimental animals. Clark et al. (1981, 1983) have reported that in mice even a cumulative dose of as low as 4 ng/kg TCDD (weekly injections of 1 ng/kg over a 4-week period) results in a significant impairment of the generation of cytotoxic T lymphocytes in response to allogeneic cells. If confirmed, this finding will be of significance in determining accurate no observed effect levels.

A common feature of exposure to immunotoxic PHH is long-lasting thymic atrophy (Kerkvliet 1984). In addi-

tion, there is mounting evidence for a causal relationship between the molecular structure of PHH, activation of an aromatic hydrocarbon gene complex and immunotoxic activity. The aromatic hydrocarbon (Ah) gene complex in mice is inherited as an autosomal dominant trait (Nebert et al. 1972) and controls responsiveness to induction of cytochrome P-448-dependent enzymes, of which aryl hydrocarbon hydroxylase (AHH) has been most extensively studied. A putative gene product of the Ah gene complex is a cytosolic receptor protein which binds stereospecifically with certain PHH congeners (Poland et al. 1976). It is currently believed that in susceptible strains, following binding, the receptor-ligand complex translocates to the nucleus (Greenlee and Poland 1979; Okey et al. 1979, 1980), interacts with DNA (Carlstedt-Duke et al. 1981) and results in gene activation and the induction of monoxygenase activity in sensitive tissues, including the thymus.

Experiments with hybrids and backcrosses have shown that TCDD-mediated immunosuppression correlates closely with AHH inducibility (Silkworth and Vecchi 1985). Furthermore, investigations with a series of PCB isomers have demonstrated that immunotoxic effects were found in C57BL/6J (Ah^b) but not DBA/2J (Ah^d) mice (Silkworth and Vecchi 1985). In most cases the immunotoxicity of PHH is associated with thymic atrophy in addition to AHH induction. However, functional immunosuppression can occur in the absence of morphological changes in the thymus (Silkworth and Vecchi 1985). Collectively, these data suggest that PHH-mediated effects on the immune system are related to the Ah gene complex and the induction of microsomal enzymes. A critical lesion in the immune system has yet to be defined and it is worth emphasising that reduced thymic cellularity and metabolic alterations following induction of hepatic and extra-hepatic enzymes may play roles of some importance in effecting changes in immune status.

While there is compelling evidence that TCDD and other halogenated aromatic hydrocarbons are immunotoxic in rodents, the majority of data available from the examination of dioxin-exposed individuals might be interpreted to suggest that man is less susceptible to TCDD-induced changes in immune competence (Reggiani 1978; Knutsen 1984). Such an assumption is not unreasonable, as extensive inter-species differences in susceptibility to TCDD-induced toxicity have been reported. A cautionary note is, however, appropriate as the route and level of exposure to TCDD in subjects studied immunologically was not determined. Moreover, studies of rats and mice have revealed that the developing immune system of the foetus and newborn is particularly susceptible to the immunotoxic effects of TCDD (Vos and Moore 1974).

In the case of polybrominated biphenyls, however, there are some reports that exposure is associated with measurable changes in human lymphocyte function (Bekesi et al. 1978, 1983). Also, there exists evidence that individuals exposed to polychlorinated biphenyls through consumption of contaminated rice oil exhibit various immunological abnormalities (Chang et al. 1981, 1982a, b; Lee and Chang 1985) and an increased susceptibility to respiratory infection (Shigematsu et al. 1978).

Organotins. The long chain dialkyltin compounds, in particular di-*n*-butyl (DBTC) up to di-*n*-octyltin dichloride (DOTC), have been shown to cause thymic atrophy (Seinen and Willems 1976; Seinen et al. 1977a; Miller et

al. 1984) and immune dysfunction (Seinen et al. 1977b; 1979). Although the mechanism(s) through which dialkyltin compounds influence the thymus are not fully understood, it has been possible to exclude indirect effects resulting from either stress-related increases in corticosteroids (Seinen and Willems 1976) or diminished somatotrophic hormone release (Penninks and Seinen 1985). The suggestion is that dialkyltins exert a direct anti-proliferative effect on lymphocytes: a view supported by in vitro studies (Penninks and Seinen 1987). To our knowledge the influence of organotins on the human immune system has not, as yet, been studied.

Heavy metals. In recent years there has been an increasing interest in the possible immunotoxic properties of heavy metals. The most thoroughly studied metal in this respect is lead. Since the first reports implicating lead as an immunotoxin (Koller 1973; Koller and Kovacic 1974) there have been a number of confirmatory studies (Koller et al. 1976; Luster et al. 1978a; Gaworski and Sharma 1978; Faith et al. 1979; Neilan et al. 1983). In fact it has been suggested that exposure of rodents to lead may result in an impaired capacity to resist bacterial or viral challenge (Hemphill et al. 1972; Gainer 1977). It is worth emphasising, however, that in some rodent studies lead was found to have no influence on (Lawrence 1981; Kimber et al. 1986a), or to enhance (Kerkvliet and Baccher-Steppan 1982), various immune functions. Preliminary results suggest that lead fails to markedly influence immune activity in man. Thus, Kimber et al. (1986b) observed that individuals chronically exposed to inorganic lead, and who exhibited blood lead levels comparable to those previously associated with immunotoxicity in rodents, possessed normal cellular immune function and plasma immunoglobulin concentrations.

Host resistance models

Host resistance models in rodents presently provide the only sure method for examining the influence of xenobiotics on the functional integrity of the immune system and its ability to efficiently eliminate pathogenic micro-organisms and tumour cells. The development of such models also provides an opportunity to assess directly the functional reserve of the immune system. It is apparent from clinical data and experimental studies that impairment of particular components of the immune system is characteristically associated with increased susceptibility to different types of infectious disease. Thus, for instance, challenge of mice with *Listeria monocytogenes* has been found to provide a reproducible method for detecting altered T lymphocyte or macrophage function (Dean et al. 1980; Vos et al. 1984). In contrast, resistance to certain transplantable tumour cells has been correlated with natural killer (NK) cell function (Dean et al. 1987).

The preliminary data available suggest that, although there is some variation between resistance models, the immune system has a significant functional reserve which must be overcome before changes in susceptibility to infection or tumour development are observed (Dean et al. 1987). Clearly there is a need for more detailed studies to provide the information required for accurate interpretation of immune function studies and assessment of toxicological significance. A cautionary note is necessary, how-

ever, as the functional reserve of the immune system may itself be variable. Many factors, including age and nutritional status, have considerable influence on immunological competence and insults to the immune system which are of little clinical significance in the young, healthy adult may be of greater importance in infants, the aged or the chronically sick.

Adverse immune reactions induced by chemicals

The directly immunosuppressive effects of xenobiotics or their metabolites have to be distinguished from their sensitising effects which may lead to *autoimmunity* and *allergy*. As reviewed above, apart from the intentional immunosuppression caused by immunomodulatory drugs, at present there is little clear evidence, albeit much concern, that xenobiotics cause unintended functional immunosuppression in man. By contrast, allergy and autoimmunity are well documented, frequent and often serious events in humans exposed to chemicals, especially drugs (DeSwarte 1986).

Autoimmunity versus allergy

Although the present report is confined to chemically induced autoimmunity, it should be emphasised that the latter shares a number of features with allergic responses to chemicals. Firstly, in both allergy and autoimmunity the immune system is stimulated to specific responses that are harmful to the body. Secondly, there are very strong effects of genetic factors predisposing to both allergic and autoimmune reactions to chemicals. In genetically susceptible individuals, even trace amounts of a chemical can elicit an adverse immunological response, whereas genetically resistant individuals will tolerate much higher doses of the chemical without showing any adverse effects. Often, these genetic effects are so strong that, when studying a mixed population consisting of susceptible and resistant individuals, the false impression may arise that there are no dose-effect relationships as far as allergic and autoimmune reactions to a chemical are concerned. Such relationships do become evident, however, when the susceptible population alone is studied.

Allergic and autoimmune reactions to chemicals can be *distinguished* as follows: in allergy, the adverse immune response is restricted to the offending exogenous agent present in the tissue. In chemically-induced autoimmunity, by contrast, the adverse immune response is not restricted to the chemical compound inducing it, but involves responses to self-antigens as well. If the inducing agent is a nonspecific immunostimulator, the adverse immune response may not be directed toward the inducing agent at all, but be confined to anti-self responses.

Classification of adverse immune reactions by their effector mechanisms

In 1963, Gell and Coombs classified adverse immune effector mechanisms into four basic types; these are shown in Table 2, together with some modifications and additions. While Table 2 only gives examples of adverse immune reactions, and only those induced by chemicals, it should be realised that protective immune reactions, e.g. those directed against infectious agents, use exactly the same effector mechanisms. With the exception of type IV

262

Table 2. Classification of the effector mechanisms of adverse immune reactions[a]

Gell and Coombs 1963	Immune effector mechanisms		
	Designation	Principal components	Examples of harmful tissue reactions
—	Neutralisation	Antibody	Insulin resistance, pernicious anaemia, myasthenia gravis
Type I	Anaphylactic, reaginic, immediate-type hypersensitivity	IgE antibody, mediators released from mast cells	Asthma, urticaria, allergic shock, hay fever
Type II	Cytotoxic	Antibody	Haemolysis, leukopenia, thrombocytopenia[b]
Type III	Immune complex	Antigen-antibody complexes	Vasculitis, glomerulonephritis, serum sickness, SLE
Type IV	Delayed hyper-sensitivity	T cells and macrophages	Contact dermatitis, berylliosis

[a] Taken from Sell (1987) and modified
[b] There is evidence that in certain patients the immunologically mediated cytopenias are mediated by excessive activity of T suppressor cells rather than antibody

reactions, all the effector mechanisms listed in Table 2 are antibody mediated. It can hardly be overemphasised, however, that in the vast majority of cases it is the T lymphocyte that determines whether or not antibody is produced.

Definition and classification of autoimmune diseases

Autoantibodies, the products of autoreactive B lymphocytes, occur in most of the autoimmune diseases (Table 3). In some of these conditions it is the autoantibodies themselves that directly cause the pathological changes. Examples here are the autoantibodies directed against the acetylcholine receptor which cause myasthenia gravis, those directed against red cells which cause autoimmune haemolytic anaemia, and those directed against the glomerular basement membrane which cause Goodpasture's syndrome. In other autoimmune diseases, however, autoantibodies are associated with the disease and indicate pathological damage, but alone may fail to initiate the disease process. Instead, the initial lesion may be partly or entirely caused by cellular immune mechanisms. Examples of the latter are Hashimoto's thyroiditis which is associated with autoantibodies against thyroglobulin and other thyroid antigens, and juvenile diabetes mellitus which is associated with autoantibodies against a cytoplasmic component of pancreatic islet cells. Thus, the appearance of serum autoantibody does not necessarily imply the existence of autoimmune disease. For instance, antinuclear autoantibodies occur in a proportion of healthy middle-aged women, and, with aging, rheumatoid factors (autoantibodies to immunoglobulin) as well as antinuclear autoantibodies occur with increasing frequency without a concomitant autoimmune disease. In these cases, however, the autoantibodies usually are of low titre and, probably, low affinity. On the

other hand, autoantibodies may be the first indicators of an autoimmune disease that becomes overt only months or years thereafter.

A convenient, albeit somewhat arbitrary, classification of autoimmune diseases divides these into organ-specific and non-organ-specific or systemic ones. *Organ-specific* autoimmune diseases are limited to a single organ, e.g. the thyroid gland in autoimmune thyroiditis, and correspondingly, the autoantibodies in these conditions are directed

Table 3. Survey of human autoimmune diseases

A. Diseases in which pathogenic autoimmune reactions are certain or likely because the self-antigens involved have been relatively well defined

Disease	Self-antigens (as defined by the autoantibodies involved)
Autoimmune chronic active hepatitis, virus-negative	Membrane and microsomes of liver cells including P-450 cytochrome isoenzymes
Autoimmune haemolytic anaemia	Membrane components of erythrocytes
Bullous pemphigoid	Basement membrane of skin
Goodpasture's syndrome (glomerulonephritis and alveolitis with linear immunoglobulin deposits along the glomerular and alveolar basement membranes)	Components of the glomerular basement membrane (GBM) and alveolar BM
Guillain-Barré syndrome	Myelin and other components of the sheets of peripheral nerves
Hashimoto's thyroiditis	Cytoplasmic or microsomal thyroid antigen, thyroglobulin
Idiopathic leucocytopenia	Membrane components of leucocytes
Idiopathic thrombocytopenia	Membrane components of platelets
Male infertility (certain cases)	Spermatozoa
Myasthenia gravis	Acetylcholine receptor at the neuromuscular synapsis
Pemphigus vulgaris	Desmosomes linking epithelial cells of the skin
Pernicious anaemia	Intrinsic factor (produced by parietal cells for absorption of vitamin B_{12})
Primary Addison's disease	Microsomal antigens in the adrenal cortex
Progressive systemic sclerosis (scleroderma)	Various antigens in cell nuclei, especially nucleoli
Systemic lupus erythematosus (SLE)	Various nuclear antigens, especially double-stranded DNA; antigens on leucocytes and erythrocytes
Thyrotoxicosis	TSH receptors
Wegener's granulomatosis (inflammatory disease of veins and arteries, especially in the lung and kidneys)	Alkaline phosphatase-like material on endothelial cells and neutrophils (Lockwood et al. 1987)

Table 3. (Continued)

B. Diseases with immunological pathogenesis in which, however, the relevant self-antigens are less well defined or unknown (in the latter case it cannot be excluded that the relevant antigens are foreign structures implanted into the tissue)

Disease	Suspected self-antigens
Glomerulonephritis with granular deposits of immuno-globulin along the glomerular basement membrane (GBM)	1) Antigens from the circulation, such as DNA, which have been implanted along the GBM 2) Autochthonous membrane structures on the epithelial cells covering the GBM, e.g. glycoproteins belonging to the gp 330 family
Juvenile diabetes mellitus (type I diabetes)	Antigens of the insulin-producing beta cells in the pancreas
Rheumatoid arthritis	Antigens of articular cartilage, such as chondroitin sulphate and a core protein of proteoglycan: collagen: IgG
Morbus Sjögren (lymphocytic infiltration of salivary and lacrimary glands)	Unknown
Polymyositis (lymphocytic infiltration of striated muscles)	Unknown
Dermatomyositis	Unknown
Lichen ruber	Unknown
Uveitis, certain types	Unknown antigens of the ocular lens
Primary biliary cirrhosis	Inner mitochondrial membrane
Ulcerative colitis	Unknown
Multiple sclerosis	Unknown

towards tissue-specific antigens, such as thyroglobulin. *Non-organ-specific* autoimmune diseases, by contrast, involve autoantibodies to ubiquitous self-antigens and, in a varied pattern of expression, affect several organs. The autoantibodies most frequently looked for in the laboratory investigation of these patients are antinuclear antibodies and rheumatoid factors. An example of a non-organ-specific autoimmune disease is systemic lupus erythematosus (SLE) in which high titres of IgG autoantibodies to a variety of nuclear antigens, in particular double-stranded DNA, are produced; this results in the formation of immune complexes which are deposited in the vessel walls and along basement membranes of different tissues and thus cause pathological alterations. Other examples of non-organ-specific autoimmune diseases are rheumatoid arthritis, Sjögren's syndrome, dermatomyositis, and scleroderma (progressive systemic sclerosis). Histologically, all these diseases are characterised by inflammatory lesions of the blood vessels and fibrinoid degeneration in the connective tissue; therefore, this family of diseases is also termed collagen-vascular diseases.

A given patient may have more than one autoimmune disease. Such an overlap is seen rather often among the organ-specific diseases on the one hand and among the

non-organ-specific diseases on the other hand. There is hardly any overlap, however, between the organ-specific and the non-organ-specific groups of autoimmune disease, which suggests a difference in their pathogenesis (cf Fig. 2 *A* and *B*).

Role of T cells and molecules of the major histocompatibility complex (MHC) in the immune response

T lymphocytes play a central regulatory role in the immune response to both foreign antigens and self-antigens. T cells can be divided into at least two functional subclasses, T helper/inducer (Th) and T killer/suppressor cells. T cells recognise antigen via a specific two-chain receptor on their surface.

Upon stimulation by specific antigen, Th cells secrete various interleukins, which act on other cell types and induce them to perform their specific functions. One of the interleukins produced by Th cells is interleukin 2 (IL-2) which acts mainly on other T cells, especially T killer cells. Another, interleukin 4 (IL-4), induces proliferation of haemopoietic cells, mast cells and B cells; in addition, IL-4 enables B cells to switch from the production of the relatively T cell-independent IgM antibody isotype to that of antibodies of the IgG and IgE isotype. Th cells also play a central role in the development of cellular immune (type IV) reactions (Table 4C). T killer cells, by contrast, are effector cells themselves in that they directly kill target cells carrying foreign antigen on their surface.

Unlike B cells which by their specific immunoglobulin receptors can recognise and bind soluble antigen, T cells can recognise antigen only if it is presented to them on the surface of another cell in conjunction with structures of the MHC. While T killer cells recognise antigen in conjunction with class-I MHC structures, Th cells recognise antigen in conjunction with class-II MHC structures. Small chemicals, such as trinitrophenyl (TNP), can be directly coupled by covalent bonds to the cell membrane and thus be recognised by specific T cells. It is not clear whether chemicals have to directly bind to MHC structures in order to become immunogenic for T cells.

In both man and laboratory animals there is a tremendous genetic polymorphism of the MHC. A functional consequence of this polymorphism is that a given antigen A may adequately be presented to T cells by the product of an assumed MHC allele[h], but inadequately by that of an assumed MHC allele[i]. Therefore, individuals possessing MHC alleles[h] are high responders with respect to antigen A, whereas individuals possessing MHC alleles[i] are low responders. However, an MHC high responder to antigen A may well be a low responder to the structurally different antigen B, and vice versa. In other words, the action of MHC structures is selective. The human MHC, HLA, codes for the class-I molecules A, B and C and the class-II molecules DR, DP and DQ. The products of different HLA alleles are designated by numbers, e.g. *HLA DR3*.

Role of T cells, B cells and MHC molecules in autoimmunity

During ontogeny the immune system acquires tolerance to self-antigens while developing the capacity to defend the body against foreign antigens. This distinction between "self" and "non-self" is an unique feature of the immune system and is crucial for the maintenance of health. Self-tolerance does not imply, however, that potentially auto-

Table 4. Examples of adverse immunological side-effects of drugs in man[a,b]

A. Drug-induced autoantibodies

Disease	Inducing drug
Autoimmune chronic active hepatitis, virus-negative	Halothane, tienilic acid
Autoimmune haemolytic anaemia, certain types	a-Methyldopa, L-dopa, captopril, cefalexin, mefenamic acid, penicillins
Goodpasture's syndrome	D-penicillamine
Granulocytopenia, certain types	Aminopyrine, captopril, cefalexin, chloral hydrate, chlordiazepoxide, chlorpromazine, chlorpropamide, gold salts, mercurial diuretics, indomethacin, p-aminosalicylic acid, penicillins, sulphapyridine/sulphathiazol, thiouracils, tolazoline
Myasthenia gravis	D-penicillamine, possibly gold salts
Phemphigus vulgaris	D-penicillamine
Bullous pemphigoid	D-penicillamine
SLE	Gold salts, griseofulvin, hydralazine, phenytoin, practolol, D-penicillamine, procainamide, thiouracil, and others
Immune complex type glomerulonephritis	Gold salts, D-penicillamine and other drugs with an -SH group

B. Drug-induced immunological diseases with unknown pathogenesis

Aplastic anaemia, certain types	D-penicillamine, phenytoin, quin-acrine, oxyphenyl, phenylbutazone
Intrahepatic cholestasis/cholangitis	Chlorpromazine, chlorpropamide, erythromycin estolate, imipramine, nalidixic acid, nitrofurantoin
Hepatitis, non-viral	Aminosalicylic acid, amiodarone, captopril, isoniazid, phenytoin and other hydantoins, and others
Hypogammaglobulinaemia	Gold salts, phenytoin
Infectious mononucleosis-like syndrome	Aminosalicylic acid, dapsone, phenytoin
Interstitial nephritis	Azathioprine, cephalosporins, furosemide, penicillins (esp. methicillin), phenindione, phenytoin, rifampicin, sulfinpyrazone, sulphonamides, thiazides, thiouracil
Lymphadenopathy/(pseudo) lymphoma/M. Hodgkin	Phenytoin and other hydantoins, possibly gold salts
Peripheral neuritis	Colchicine, gold salts, nitrofurantoin, sulfonamides
Serum sickness	Penicillins, cephalosporins, streptomycin, sulphonamides, and others
Skin: immunological drug reactions can mimic virtually all clinical and histological patterns of disease	Antibiotics, barbiturates, diuretics, gold salts, hydantoins, tranquilisers, and many others

Table 4. (Continued)

Disease	Inducing drug
Thrombocytopenia, certain types	Acetazolamide, acetylsalicylic acid, carbamazepine, cephalothin, chloramphenicol, digitoxin, gold salts, imipramine, levodopa, meprobamate, methyldopa, para-aminosalicylic acid, phenylbutanzone, phenytoin, quinidine, quinine, rifampicin, spironolactone, stibophen, sulphonamides, sulphonylureas, thiazides
Vasculitis, different types	Allopurinol, busulfan, indomethacin, isoniazid, iodides, penicillin, phenothiazines, phenylbutazone, tetracyclines, thiazides, thiouracils

C. Examples of allergic reactions to chemicals (only reactions against non-self antigens are involved)

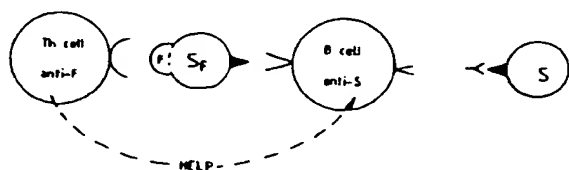|	
Allergic asthma and related conditions	Different types of allergen inhaled at the work place, such as the dust of manufactured antibiotics, ethylenediamine, formaldehyde, insecticides, isocyanates, salts of the heavy metals chromium, cobalt, mercury, nickel, platinum
Contact dermatitis	1) Very many topically applied drugs, such as antibiotics, antihistamines, local anaesthetics 2) A great variety of other chemicals, including many different organic compounds and the salts of heavy metals, such as chromium, cobalt, mercury, and nickel
Food allergy	Many different types of food additive, chemical contaminations of food

[a] Compounds are listed alphabetically and not according to the frequency of adverse immunological side-effects they induce
[b] Major sources of reference: Aaronson (1980), DeSwarte (1980), Parker (1980)

active B cells and T lymphocytes are all absent from the body. Instead, autoreactive B cells (Dighiero et al. 1983) and T cells (Cohen 1986) do occur in normal individuals, but they are held in check by mechanisms that are incompletely understood. Self-tolerance at the level of T cells, however, appears to be much tighter than at the level of B cells; this is important with respect to the T-cell by-pass concept of autoantibody formation (see below). Moreover, while the existence in normal individuals of B cells capable of producing autoantibodies to self-antigens circulating at low concentration, such as DNA, has unequivocally been demonstrated (Dighiero et al. 1983), it is not clear whether this is true for all types of self-antigen, e.g. those circulating at high concentration, such as serum albumin. In normal individuals, potentially autoreactive lymphocytes are completely or largely inactive, but under certain
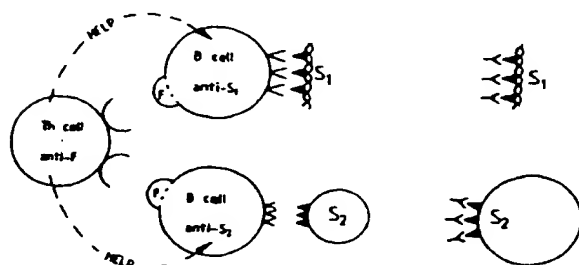
Table 5. Selected examples of HLA phenotypes and other genetically determined traits that predispose to chemically induced auto-immune diseases in man

Disease	Aetiologic agent	Predisposing genetic factors		Authors
		HLA	Other	
Myasthenia gravis	D-penicillamine (antirheumatic drug)	DR1, Bw35	slow sulphoxidisers	Dawkins et al. 1982; Emery et al. 1984; Panai et al. 1983
Glomerulonephritis due to granular IgG deposits at the basement membrane, proteinuria	D-penicillamine	DR3, DR4		Dawkins et al. 1982; Wooley et al. 1980
Glomerulonephritis due to granular IgG deposits, proteinuria	gold sodium thiomalate (antirheumatic drug)	DR3, B8'	slow sulphoxidisers	Wooley et al. 1980 Perrier et al. 1985; Hakala et al. 1986
Autoimmune thrombocytopenia	gold sodium thiomalate	DR3		Coblyn et al. 1981; Adachi et al. 1984
Drug-induced SLE	hydralazine (anthihypertensive drug)	DR4	slow acetylators, female sex	Batchelor et al. 1980
Scleroderma-like lesions (sclerosis of the skin, Raynaud's phenomenon, arthralgia and arthritis, pulmonary and portal fibrosis, thrombocytopenia)	vinyl chloride (industrial chemical)	DR5		Black et al. 1986

• While HLA DR3 and DR8 are susceptibility factors, DR7 determines resistance

hanced production in vivo of IgG antibodies (Ptak et al. 1984). Conceivably, some compounds producing autoimmunity bind to certain class-II structures better than others. Evidence for a preferential interaction of certain drugs with certain HLA class-1 structures has been obtained by Claas and van Rood (1985). These authors incubated human peripheral blood leucocytes with high concentrations of various drugs in vitro and demonstrated consistent patterns of selective blocking of certain HLA class-I structures by certain drugs.

In the experiment of Ptak et al. (1984) mentioned above, murine B cells were rendered "foreign" by TNBSA (trinitrobenzene sulfonic acid), a chemical that covalently binds to protein. Whether other chemicals, such as drugs, will spontaneously bind to the membrane of lymphoid cells in vivo and thus render them "foreign" is largely unknown. Some indirect evidence that this may, indeed, happen has been obtained from studies on the sensitization to penicillin in humans (de Weck 1983) and D-penicillamine in mice (Nagata et al. 1986). In the latter case, it was shown that even after oral application of a high dose of D-penicillamine, peritoneal macrophages of recipient mice were altered in such a way that they elicited reactions by specific Th cells (Vogeler and Gleichmann 1988). Whether this alteration is, indeed, responsible for the SLE-like autoimmune disease inducible by D-penicillamine in the rat (Donker et al. 1984) and for human autoimmune diseases induced by this drug (Table 4) is yet unknown.

Cells whose function includes presentation of antigen to Th cells constitutively express class-II MHC structures. Such cells are dendritic cells, Langerhans cells of the skin, and B lymphocytes, especially activated B cells. Many other cell types, however, including, for instance, epidermal cells, thyrocytes and pancreatic islet cells, facultatively express class-II structures during inflammatory responses in the respective tissue. This is due to the local production of immunological mediators, such as tumour necrosis factor, gamma-interferon and lymphotoxin. Inflammatory reac-

tions also include destruction of cells and hence release and/or alteration of self-antigens. These two phenomena together enhance the chance for (self-) antigen to be recognised by (autoreactive) Th cells and thus could increase the chance for development of autoimmune disease (Londei et al. 1985; Pujol Borell et al. 1987). With respect to chemically-induced autoimmunity, it would be of value to examine whether chemicals can induce the expression of class-II MHC genes.

Chemicals as aetiological agents of human autoimmune diseases

In contrast to the pathogenesis of autoimmune diseases which begins to be unravelled, as outlined above, almost nothing is known about their *aetiology*. In those admittedly few cases, however, where an aetiological agent of human autoimmune disease can be identified, most often this agent is a chemical compound, and this, in turn, most often is a drug. For three reasons it is not legitimate in the present state of ignorance to draw the converse conclusion from these observations, namely that chemicals are also the main suspects for the many cases of autoimmune disease with unknown aetiology. Firstly, there may be an observer's bias for drug-induced autoimmune diseases in that patients receiving drugs are under close medical supervision to begin with, so that chances for such cases being noticed and reported are presumably greater than with other aetiological agents. Secondly, the natural history of autoimmune diseases induced by drugs is often different from that of the same disease developing spontaneously, because drug-induced autoimmune symptoms usually disappear after withdrawal of the drug, whereas idiopathic autoimmune diseases often progress or follow a course characterised by relapses and remissions. Thirdly, it is conceivable that autoimmune diseases develop without an exogenous cause due to spontaneously arising errors in the regulation of the immune system. This possibility is sup-

ported by the fact that there are inbred strains of animals that spontaneously develop autoimmune disease.

As can be seen from Table 4, drugs can induce a great variety of organ-specific and non-organ-specific autoimmune diseases. Several drugs can induce more than one kind of autoimmune disease (see, for instance, D-penicillamine and phenytoin), and certain drugs, such as penicillin, can induce both autoimmune disease and allergy (Table 4). With the exception of adverse immunologic reactions to penicillin (de Weck et al. 1983), only very few drug reactions have been studied in a systematic fashion, however. In particular, investigations of specific anti-drug reactions of T lymphocytes are scarce. Hence, in most instances of drug-induced autoimmunity it has not been proven if, indeed, Th are involved in the pathogenesis, as one would postulate on theoretical grounds. (A reaction of Th cells against the drug does not preclude, of course, that antibodies to that drug may also be produced.) It is also unclear, in the vast majority of cases, whether an adverse immunological reaction to a given drug is elicited by the compound or a metabolite.

A provocative, albeit indirect, observation for the involvement of drug metabolites in human autoimmune disease has recently been reported by Beaune et al. (1987). They showed that patients who developed a nonviral hepatitis after treatment with tienilic acid had IgG autoantibodies directed against the isoform P-450-8 of cytochrome P-450 obtained from human liver microsomes. These autoantibodies specifically inhibited the hydroxylation of tienilic acid by human liver microsomes. The authors suggest that cytochrome P-450, originally present in the endoplasmic reticulum of the hepatocyte, could be alkylated by a reactive metabolite and migrate onto the hepatocyte membrane surface. At this level, the modified protein could be recognised by specific Th cells reacting to that part of the molecule derived from the reactive metabolite. These functionally active Th cells, in turn, would allow formation of IgG autoantibodies that recognise the native protein (cf Fig. 2A). One might add that, in retrospect, formation of autoantibodies to cytochrome P-450 is perhaps not totally surprising because this enzyme system generates highly reactive metabolites from a variety of different parent compounds. Prior to metabolic transformation, the compounds usually are not very reactive and, hence, not, or hardly, immunogenic. Thus, in an immunological sense this particular enzyme system marks a strategic borderline between self and non-self.

Genetic factors predisposing to human autoimmune diseases induced by drugs or occupational chemicals

It is clear from the few studies performed that both immunological and pharmacological genetic traits can be involved in the development of drug-induced autoimmunity in man. As an immunogenetic factor, the HLA alleles of the respective patients were determined, while the pharmacogenetic traits studied were those that are relevant for the metabolism of a particular drug (Table 5).

In contrast to the autoimmunising side-effects of drugs which are well documented, little is known about the auto-immunising potential of occupational and environmental chemicals. Severe scleroderma-like lesions have been reported in workers exposed to vinyl chloride (Table 5) as well as workers exposed to quartz (Ziegler et al. 1986). Moreover, scleroderma-like lesions, but also SLE, have

been reported in women carrying silicon-containing breast protheses (Kumagai et al. 1984; Guillaume et al. 1984). An SLE-like disease can also develop in humans exposed to a food additive, azodyl tartazine, or the industrial chemical hydrazin, and in monkeys and humans fed an amino acid present in alfalfa seeds (Pereyo 1980; Malinow et al. 1982; Reidenberg et al. 1983)..

Mercurials as aetiological agents of autoimmunity and increased IgE production

A chemical which induces autoimmunity and has been studied in some depth is mercury. In several rodent species, mercurials have been shown to cause an SLE-like autoimmune syndrome as well as a marked increase in IgE formation. A prominent feature of the mercury-induced autoimmune syndrome in rodents is glomerulonephritis, and this disease at least has also been documented in humans exposed to mercurials (Table 6). The observations in man were made in cases of mercury poisoning or exposure to mercury as a constituent of drugs or cosmetics. In all these cases, subjects were exposed over a relatively short period of time to high concentrations of mercury. While it is unknown whether or not the concentrations of mercury existing at the work place and in the environment constitute a risk with regard to immunopathology, it is noteworthy that the immunopathological signs inducible by mercury are not confined to certain mercury compounds or routes of administration of such compounds (Table 6). Moreover, the dosages of $HgCl_2$ that induce autoimmune disease and enhanced IgE formation in rodents are clearly below the dose range in which general toxicity is observed.

The pathogenesis of mercury-induced autoimmunity has been analysed in detail in the rat. In this species, there is a stringent genetic control of $HgCl_2$-induced autoimmunity and increased IgE production which is determined by three to four independently segregating loci. One of these loci segregates with the MHC and exerts a strong effect, the others have not been identified. Of 22 inbred strains of rat studied, the Brown Norway strain was the most susceptible, because it developed all the symptoms listed in Table 6; other strains were partially susceptible, and yet others, such as Lewis, were resistant. In Lewis rats, even doses of $HgCl_2$ which induce acute tubular necrosis failed to induce autoimmune phenomena.

All autoimmune phenomena induced by $HgCl_2$ are T cell dependent, since they fail to develop in $HgCl_2$-treated athymic rats. The pathological alterations of mercury-induced autoimmunity resemble those of chronic GVH disease (GVHD) (Druet et al. 1987). Chronic GVHD is caused by an excessive activation of Th cells which, secondarily, activate other immune cells, in particular B cells; the latter then produce antibodies, especially SLE-like autoantibodies of the IgG isotype (Gleichmann et al. 1984). In mercury-induced autoimmunity, too, there is an excessive activation of Th cells. This suggests that there is a common final pathway leading to SLE-like autoimmunity in chronic GVHD and mercury-induced autoimmunity.

The popliteal lymph node assay (PLNA) in rodents, a test predicting the sensitizing and/or immunostimulatory potential of xenobiotics

The central role of T cells in the initiation of both autoimmunity and allergy has been emphasised. Hence, in the

Table 6. Survey of autoimmune and other immunopathological alterations induced by exposure to mercury compounds of humans and genetically susceptible strains of rat, respectively

Species studied	Mercury compounds studied	Route of application	Pathological alteration induced	Selected references
Man[a]	Various Hg-containing drugs and ointments	Oral, percutaneous, and various routes of injection	Membranous glomerulonephritis with granular IgG deposits in the mesangium and at the glomerular basement membrane	Fillastre et al. 1984; Tubbs et al. 1982
			Contact dermatitis and other forms of dermatitis	Taugner and Schütz 1966
			Enhanced IgE formation in vitro	Kimata et al. 1983
Rat[a,b]	$HgCl_2$, CH_3HgCl, Hg-containing drugs and ointments	Inhalative, intraperitoneal, oral, percutaneous, subcutaneous	Lymphadenopathy, splenomegaly	Druet et al. 1983, 1987
			Lymphocytic infiltration of salivary glands (similar to M. Sjögren)	Aten and Weening 1985
			Intravascular blood coagulation	Druet et al. 1983
			IgG autoantibodies against the glomerular basement membrane (similar to Goodpasture's syndrome)	Druet et al. 1983, 1987
			Membraneous glomerulonephritis with granular IgG deposits in the mesangium and at the glomerular basement membrane	Druet et al. 1983, 1987; Weening et al. 1981
			IgG deposits in the walls of blood vessels	Druet et al. 1983, 1987
			IgG autoantibodies to various nuclear antigens and other autoantibodies	Weening et al. 1981
			Polyclonal B cell stimulation	Druet et al. 1983, 1987
			Extreme increase in total serum IgE and, if antigen is administered simultaneously, formation of specific IgE antibodies	Druet et al. 1983, 1987

[a] Some of the abnormalities listed above could also be induced in rabbits and guinea pigs exposed to $HgCl_2$ (Polak et al. 1968; Albini et al. 1983)

[b] Almost all the abnormalities induced in the rat can also be induced by $HgCl_2$ administration to susceptible strains of mouse (Fleuren et al. 1985; Robinson et al. 1986; Hultman and Enestrom 1987; Mirtschewa et al. 1987; Pietsch et al. 1987)

preclinical test phase there is a need for simple laboratory tests to predict the immunogenicity of chemicals for T cells. The PLNA in laboratory rodents might suit this purpose. The scheme of the PLNA is shown in Fig. 3.

A survey of results obtained by the PLNA is given elsewhere (Gleichmann et al. 1988; Gleichmann and Klinkhammer 1988; Kammüller and Seinen 1988; Kammüller et al. 1988; Stiller-Winkler et al. 1988). During the primary PLN response to small chemicals or conventional antigens, the maximal response in the mouse hardly exceeds a PLN weight index of 10. In the rat, however, the values of PLN indices are usually higher than in the mouse. With most chemicals tested the primary PLN response, if inducible, peaked within the first 10 days after injection and returned back to normal at week 3–4; exceptions from this rule were seen with high dosages of heavy metals ($HgCl_2$, $CdCl_2$) and, in particular, quartz.

Quartz induced an ever progressing PLN enlargement so that after 6 months the weight increase of the draining PLN had reached values of up to 200 times that of the contralateral PLN (Stark et al. 1988). Quartz also was exceptional in that the lymph node enlargement induced did not

require presence of T lymphocytes (Zaidi et al. 1979); this fits the notion that quartz (SiO_2) is not an antigen. Quartz is phagocytosed by macrophages and, since it is undigestible, persistently activates these cells to release factors which, secondarily, activate lymphocytes. Thus, in the case of quartz the PLNA reflects non-specific immunostimulatory properties of the test compound.

By contrast, in all other instances where this was tested (phenytoin, D-penicillamine, streptozotocin, captopril, $AuCl_3$) the PLN response was not only T cell-dependent, but also specific. Specificity was proven by the fact that primed mice showed an enhanced PLN response upon a second injection of the same, but not another compound (Hurtenbach et al. 1987; Klinkhammer et al. 1988).

Injection of chemicals into a hind footpad is an artificial manoeuvre designed to detect the sensitizing potential of chemicals. In real life, chemicals are taken up by different routes, such as the oral and the inhalative routes, so that the mesenteric and mediastinal lymph nodes and the spleen, respectively, are the candidate lymphoid organs that may harbour sensitized T lymphocytes. It is of great practical importance, therefore, to have a simple test sys-
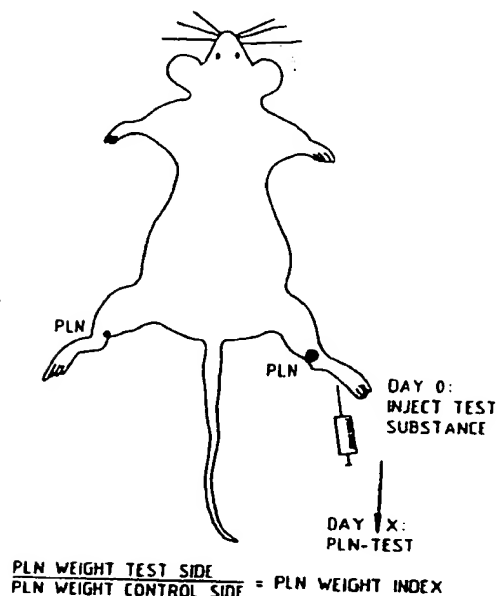
PLN WEIGHT TEST SIDE / PLN WEIGHT CONTROL SIDE = PLN WEIGHT INDEX

DAY 0: INJECT TEST SUBSTANCE

DAY X: PLN-TEST

Fig. 3. Scheme of the *direct popliteal lymph node assay (PLNA)* in rodents. The test compound is injected subcutaneously without adjuvant into one hind footpad of a test animal. The contralateral side is left untreated, or inoculated with the solvent of the test compound and thus serves as an internal control. Days thereafter, an ensuing immune reaction can be assessed by removing the PLNs and determining either the PLN weight index, or, more sensitively, the number of cells in the PLNs, or [³H]thymidine incorporation into the PLNs. PLN cells may also be analysed by flow cytometry

SPLEEN CELL DONOR
- TREATED WITH 40mg STZ/KG BW OR SOLVENT ON 5 CONSECUTIVE DAYS
- TRANSFER AFTER 4 WKS

RECIPIENT

01mg STZ OR ALLOXAN OR SOLVENT AT - 24h

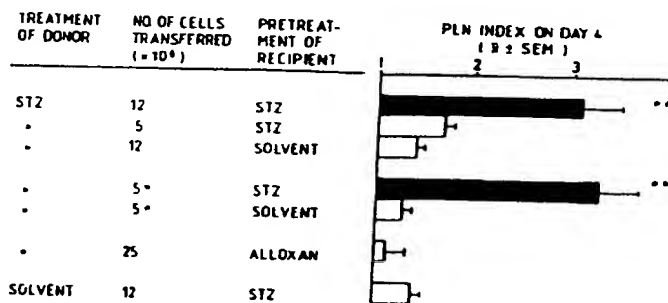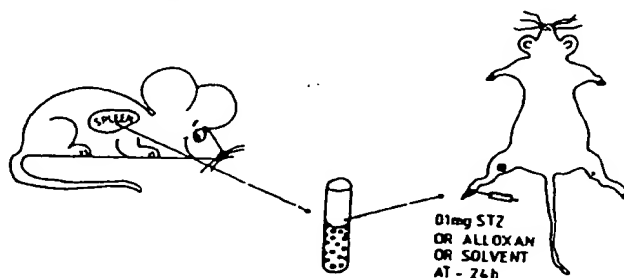| TREATMENT OF DONOR	NO OF CELLS TRANSFERRED (=10⁶)	PRETREAT-MENT OF RECIPIENT	PLN INDEX ON DAY 4 (x ± SEM)
STZ	12	STZ	
•	5	STZ	
•	12	SOLVENT	
•	5•	STZ	
•	5•	SOLVENT	
•	25	ALLOXAN	
SOLVENT	12	STZ	

Fig. 4. Experimental design and results of the *adoptive transfer PLNA*. Mean values SEM of the PLN weight index are shown. Spleen cells of BALB/c donor mice, which had received five i.p. injections of either streptozotocin (STZ) or the solvent of STZ, were inoculated s.c. into one hind footpad of syngeneic mice. The recipients had received an s.c. injection of either a sub-immunogenic dose (0.1 mg) of STZ or alloxan or solvent into the same footpad 24 h before the cell transfer. Groups of seven mice each were used as recipients. • = enriched for T cells; •• = $p < 0.005$ versus controls. Reproduced from Klinkhammer et al. (1988) with kind permission of the authors and the American Diabetes Association Inc.

tem in which mesenteric, mediastinal or splenic T lymphocytes from rodents, which received a chemical in the context of routine toxicity testing, can be assayed for possible sensitization to that chemical. Such a test system has recently been established in the form of the adoptive transfer PLNA (Fig. 4). Potentially sensitised T cells were taken from donor mice that had received five i.p. injections of streptozotocin and had then been rested for 4 weeks. It should be noted that by histopathological criteria, the spleens of these mice were normal. Splenic T cells obtained from such donor animals were injected into the footpad of syngeneic rodents, together with a dose of streptozotocin which by itself is too low to induce a primary PLN response. Two to five days after the cell transfer, a specific PLN enlargement was seen in the recipient. In this experiment, the recipient is rather inert in as much that it serves only to properly present the test compound to the donor's T cells and reflect (by PLN enlargement) their reaction. Thus, the adoptive transfer PLNA showed that sensitized T cells from the spleen of a donor mouse, which had been exposed to a drug by multiple i.p. injections, can be demonstrated by specific restimulation in the PLNA. The optimal conditions for the adoptive transfer PLNA need to be defined. It should be tested, for instance, if a chemical will also be recognised by the transferred memory T cells if it is administered to the prospective recipient not into the footpad along with the donor T cells, but via the route and at a dosage that represent a more realistic exposure to that chemical. This should allow measurement of T cell reactions to immunologically relevant metabolites, provided

these reach the circulation. It is mandatory, however, that test systems, such as the PLNA, be validated, before they can be used in routine toxicology.

Methods to detect adverse immunological reactions to chemicals in man

In man, tests to detect adverse immunologic reactions to chemicals are usually performed a posteriori, i.e. after a presumed sensitization to a xenobiotic. At the antibody level, a variety of serological tests to detect specific antibodies to the suspected drugs can be performed in vitro. In vivo, the scratch test is frequently used which involves topical application of the suspected drug into the dermis. The scratch test preferentially detects antibodies formed against a drug, in particular those of the IgE isotype (type I reaction). At the T cell level, chemical compounds are tested for their capacity to elicit contact dermatitis (type IV reaction).

All these tests actually measure allergic reactions, and they are thus not necessarily relevant to autoimmunity. As discussed above, it is likely that many chemicals known to cause autoimmune disease primarily trigger reactions by specific Th cells which secondarily activate autoreactive

cells, especially autoreactive B cells. Such a chemical need neither elicit contact dermatitis nor the formation of antibodies specific for that chemical, let alone specific IgE antibodies. What is needed, therefore, is a test method demonstrating specific sensitisation of human Th cells to small chemicals. In principle, such a sensitisation can be demonstrated by the lymphocyte transformation test in vitro, where peripheral blood lymphocytes (essentially T cells) are obtained from a sensitised patient and cultured in the presence of the suspected drug. A problem of the lymphocyte transformation test in its present form is, however, that it has produced many "false" negative, and also some "false" positive results. Nonetheless, the general opinion is that the accuracy of this test can be considerably improved by further standardization (Stejskal et al. 1986) and, in particular, elaboration of the conditions required for "presenting" the test compound to the responder T cells. Here, too, the question arises whether it is the mother compound or a metabolite that is seen by the sensitized T cells of a given patient. With certain drugs, evidence in favour of metabolites has been presented by Victorino et al. (1985) and Merk et al. (1988). While Merk and colleagues failed to detect T cell transformation in response to the mother compounds, they did observe T cell transformation in response to drugs that were pre-incubated with liver microsomes, as a source of cytochrome P-450. Further experiments of this type need to be performed.

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